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DEPARTMENT OF BIOMEDICAL ENGINEERING

BM3491 Biomedical Instrumentation

UNIT- V BIOCHEMICAL MEASUREMENTS

5.1 Biochemical Sensors – pH, pO_2 and pCO_2

5.1.1 What is biochemical sensors?

- Biochemical sensors are devices designed to *detect* and measure specific *biochemical substances* within a sample.
- These substances can include various molecules such as *proteins, enzymes, hormones, DNA, RNA, glucose*, and many others.
- Biochemical sensors are widely used in fields such as *medicine*, *environmental monitoring, food safety,* and *biotechnology*.

5.1.2 Why is it necessary to maintain acid-base balance in the body?

- The normal pH of the extracellular fluid lies in the range of 7.35 to 7.45, indicating that the body fluid is slightly alkaline.
- When the pH exceeds 7.45, the body is considered to be in a state of alkalosis.
- A body pH below 7.35 indicates acidosis.
- pH of blood to Deviate *by three physiological mechanisms*:

(i) buffering by chemical means,

(ii) respiration,

(iii) excretion into the urine by kidneys.

- The blood and tissue fluids contain ***chemical buffers***, which react with added acids and bases and minimize the resultant ***change in hydrogen ions***. They respond to ***changes in carbon dioxide*** concentration in seconds.
- The respiratory system can adjust ***sudden changes in carbon dioxide*** tension back to normal levels in just a few minutes. Carbon dioxide can be ***removed*** by increased ***breathing*** and therefore, ***hydrogen concentration of the blood*** can be effectively modified
- The ***kidney*** requires many hours to readjust hydrogen ion concentration by ***excreting*** highly acidic or alkaline urine to enable body conditions to return towards normal.
- Maintaining acid-base balance is essential for the proper functioning of ***enzymes, cells***, and physiological processes throughout the body.

5.1.3 Blood pH Measurement:

- The acidity or alkalinity of a solution depends on its concentration of hydrogen ions.
- Increasing the concentration of hydrogen ions makes a solution more acidic, decreasing the concentration of hydrogen ions makes it more alkaline.
- pH is thus a measure of hydrogen ion concentration, expressed logarithmically. Specifically, it is the negative exponent (log) of the hydrogen ion concentration. pH = –log (H+)
- If the number 10^{-7} represents the concentration of hydrogen ions in a certain solution, then its pH would be 7
- The normal pH of the extracellular fluid lies in the range of 7.35 to 7.45, indicating that the body fluid is slightly alkaline.
- Electrochemical pH determination utilizes the difference in potential occurring between solutions of different pH separated by a special glass membrane.

- If the pH of one of the solutions is kept constant, so that the potential varies in accordance with the pH of the other solution, then the system can be used to determine pH.
- The device used to effect this measurement is the glass electrode.

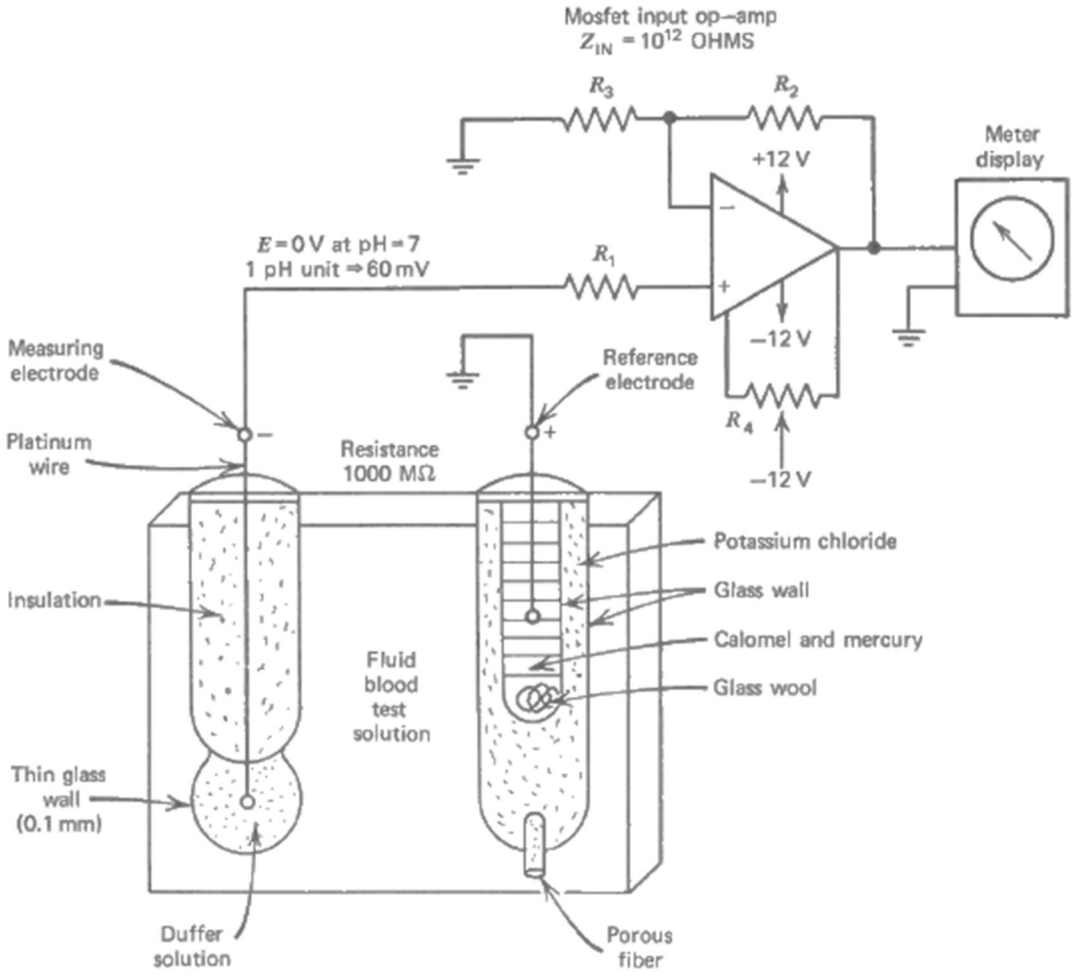


Figure. 5.1 *Basic pH glass Electrode*

- For making pH measurements, the solution is taken in a beaker.
- A pair of electrodes: one glass or indicating electrode and the other reference or calomel electrode, are immersed in the solution.
- Platinum wire immersed into a highly acidic buffer solution contained within a thin glass bulb.

- An almost universally employed reference electrode is the saturated calomel electrode (glass tube contains Potassium Chloride solution, KCL).
- When two electrodes (or one probe containing the two electrodes) are dipped into solution, some of the hydrogen ions in the solution move toward the glass electrode and replace some of the metal ions in its special glass coating.
- The platinum wire electrode generates a half-cell electrical potential that acts in combination with the stable reference calomel half-cell.
- The test solution is in common with both half-cells.
- The voltage developed across the electrodes is applied to an electronic amplifier, which transmits the amplified signal to the display.
- The resultant voltage is amplified by a high input impedance amplifier such as a MOSFET input op-amp (10^{12} Ω, differential input impedance).
- An increase in voltage means more hydrogen ions and an increase in acidity, so the meter shows it as a decrease in pH; in the same way, a decrease in voltage means fewer hydrogen ions, more hydroxide ions, a decrease in acidity, an increase in alkalinity, and an increase in pH.
- ↑ voltage = more H+ /less OH - = ↑ acidity = ↓pH
- ↓ voltage = less H+ /more OH - = ↓ acidity = ↑pH

5.1.4 Blood pO_2 Measurement:

What is Blood pO_2 ?

[Oxygen molecules dissolved in plasma (i.e., not bound to hemoglobin)]

- The *partial pressure of oxygen* (pO_2) in blood refers to the pressure exerted by oxygen gas in a mixture of gases, in this case, within the blood. It is a *measure of the amount of oxygen gas dissolved* in the blood and is usually expressed in millimeters of mercury (*mmHg*).
- The partial pressure of oxygen in the blood or plasma indicates the extent of oxygen exchange between the lungs and the blood, and normally, the ability of the *blood to adequately perfuse the body tissues with oxygen*.
- Normal arterial oxygen pressure (PO_2) is approximately 75 to 100 mmHg. When the levels drop below 75 mmHg, the condition is called ***hypoxemia.*** Oxygen at

higher than normal partial pressure leads to ***hyperoxia*** and can cause oxygen toxicity or oxygen poisoning.

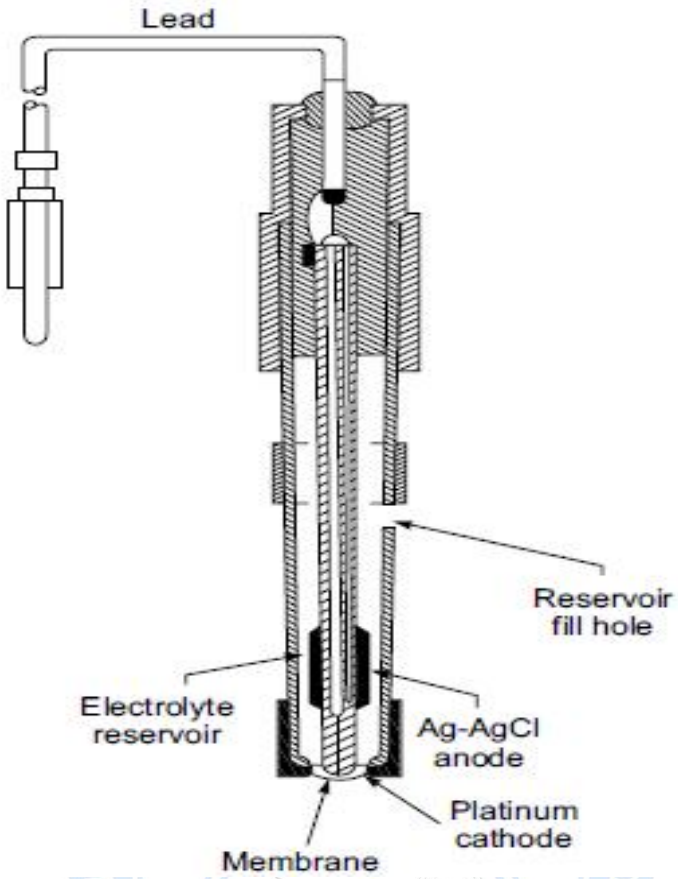
Measurement of Blood pO_2:

- The partial pressure of oxygen is usually measured with a polarographic electrode.
- A polarographic electrode, often referred to as a ***Clark electrode***, is a commonly used device for measuring the partial pressure of oxygen (pO2) in blood. It operates on the principle of ***polarography***, where the reduction of oxygen at a cathode is measured to determine the oxygen concentration.

- **Components and Operation :**

1. **Electrode Structure**:

   - **Cathode**: Typically made of platinum.
   - **Anode**: Often silver/silver chloride (Ag/AgCl).
   - **Electrolyte Solution**: Potassium chloride (KCl) solution, which surrounds the electrodes.
   - **Oxygen-permeable Membrane**: A thin membrane that allows oxygen to diffuse into the electrolyte solution while preventing the direct contact of blood with the electrodes.



Fig, 5.2: Constructional details of pO2 electrode

Fig. 15.2 shows the construction of a typical Clark-type oxygen electrode.

- The entire unit is separated from the solution under measurement by the polypropylene membrane.

2. Measurement Process:

- **Oxygen Diffusion**: Oxygen from the blood sample diffuses through the membrane into the electrolyte solution.
- **Reduction Reaction**: At the cathode, oxygen is reduced to hydroxide ions (OH-) in the presence of a polarizing voltage (typically around -0.6 to -0.8 volts).
- The overall reaction at the anode and cathode are

Cathode Reaction:

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$$

Anode Reaction:

$$4Ag^{+} \longrightarrow 4\,Ag + 4e^{-}$$

Current Measurement: The reduction reaction at the cathode produces a current proportional to the amount of oxygen present. This current is measured and converted into an oxygen concentration reading.

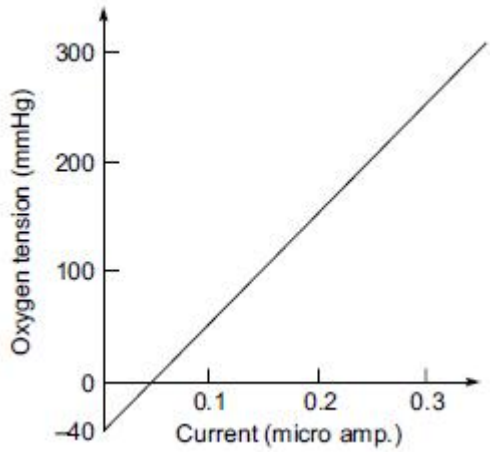


Fig. 5.3 Calibration curve of pO2 electrode

Calibration:

- For calibrating the electrode, it was necessary to know this constant for that particular electrode.
- They further showed that when the straight-line calibration curves (Fig. .5.3) were extended backwards, they did not pass through the origin, but intersected the oxygen tension axis at a negative value.
- To obtain a true zero-current (less than 10 nA), the electrolyte of the electrode is deoxygenated by bubbling nitrogen through it for about half an hour and then placing the electrode in water redistilled from alkaline pyragallol.
- The polarographic electrodes usually exhibit ageing effect by showing a slow reduction in current over a period of time, even though the oxygen tension in the test solution is maintained at a constant level. Therefore, it needs frequent calibration.

- **The principal advantages are**:

(i) ***Sample size*** required for the measurement can be extremely ***small***,

(ii) The ***current*** produced due to pO2 at the electrode is ***linearly*** related to the ***partial pressure*** of oxygen,

(iii) the ***electrode*** can be made ***small*** enough to measure oxygen concentration in highly localized areas,

(iv) the ***response time is very low***, so the measurements can be made in seconds.

- **Limitations of polarographic electrodes:**

i. The measurement of current developed at the pO2 electrode due to the partial pressure of oxygen is extremely small. The sensitivity (current per torr of oxygen tension) is typically of the order of 20 pA per torr for most commercial instruments.
ii. Subjected to Constant drift.

5.1.5 Blood pCO_2 Measurement:

5.1.5.1 What is Blood pCO_2:

- Blood pCO2 refers to the partial pressure of carbon dioxide in the blood. It is an important parameter in assessing respiratory function and acid-base balance in the body.
- **Respiration**: CO2 is a byproduct of cellular metabolism and is transported in the blood from the tissues to the lungs, where it is exhaled.
- **Acid-Base Balance**: CO2 reacts with water to form carbonic acid (H2CO3), which dissociates into hydrogen ions (H+) and bicarbonate ions (HCO3-). This reaction is crucial in maintaining the blood's pH balance.
- It is expressed in mmHg and is related to the percentage CO_2 as follows:

$$pCO_2 = \text{Barometric pressure} - \text{Water vapour pressure} \times \frac{\%CO_2}{100}$$

- At 37°C, the water vapour pressure is 47 mmHg, so at 750 mm barometric pressure, 5.7% CO2 corresponds to a pCO2 of 40 mm.

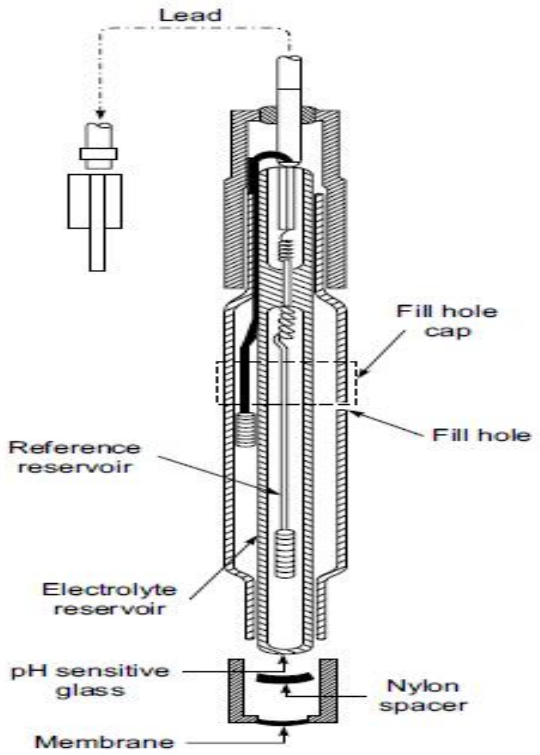


***Fig. 5.4** Construction of pCO2 electrode*

- It basically consists of a pH sensitive glass electrode having a rubber membrane stretched over it, with a thin layer of water separating the membrane from the electrode surface.
- The technique is based on the fact that the dissolved CO_2 changes the pH of an aqueous solution.
- The CO_2 from the blood sample diffuses through the membrane to form H_2CO_3, which dissociates into (H+) and (HCO–3) ions.
- The resultant change in pH is thus a function of the CO_2 concentration in the sample.
- The emf generated was found to give a linear relationship between the pH and the negative logarithm of pCO_2.

- ❒ Although the electrode could not provide sensitivity and stability required for clinical applications, it made way for realizing a direct method for the measurement of pCO2.
- ❒ The response time of the CO2 electrode is of the order of 0.5 to 3 min.

Normal Values

- **Arterial Blood**: The normal range of arterial pCO2 is approximately 35-45 mmHg (millimeters of mercury).
- **Venous Blood**: The normal range of venous pCO2 is slightly higher, typically 40-50 mmHg, due to the higher CO2 content coming from the tissues.

❒ Blood pCO2 is typically measured using a blood gas analyzer, which can be done on arterial blood samples for more accurate assessments of respiratory function and acid-base status. The Severinghaus electrode is commonly used in these analyzers to measure pCO_2.

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UNIT- V BIOCHEMICAL MEASUREMENTS

5.2 Ion selective FET (ISFET), Immunologically sensitive FET (IMFET)

5.2.1 Ion selective Field Effect Transistor (ISFET):

- An ion-selective field-effect transistor (ISFET) is a type of sensor used to *measure ion concentrations* in a solution.
- It operates on the principle of a *field-effect transistor*, where the conductance between two terminals is controlled by an electric field.
- However, in an ISFET, the gate of the transistor is a reference electrode coated with a selective membrane that responds specifically to the ions of interest.

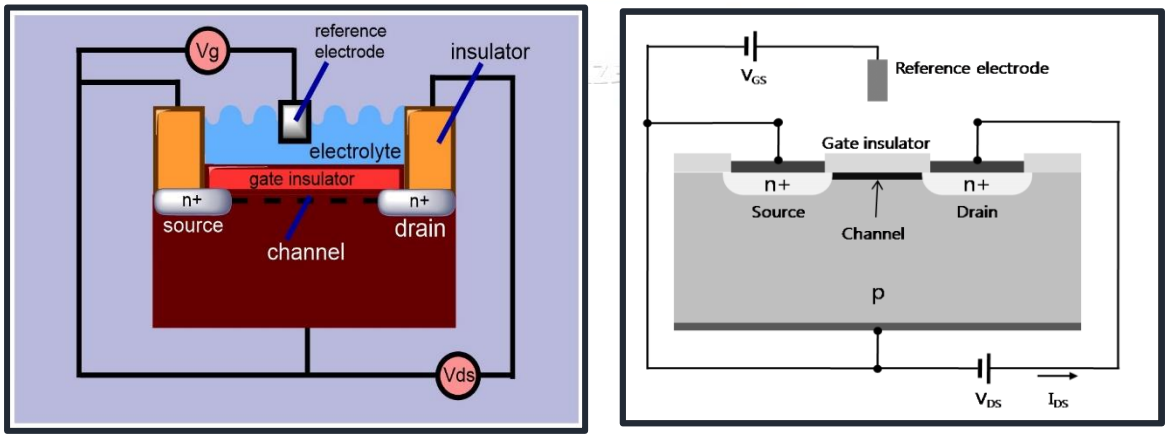


Fig. 5.2.1 Ion selective Field Effect Transistor

- Ion Sensitive Field Effect Transistor (ISFET) is in fact a *Metal Oxide Semiconductor Field Effect Transistor (MOSFET)* in which *metal gate* is *replaced by a complex structure* sensitive to hydrogen ion concentration.
- It is obvious that ISFET is obtained by replacing the standard metal gate of a MOSFET with a reference electrode, a chemically sensitive insulator between which presents a measured electrolyte.
- *The gate voltage is applied to the reference electrode* and the electrolyte closes the electric Gate-Source circuit.
- ISFET is therefore fundamentally a MOSFET and hence the theoretical description of MOSFET is essential to describe ISFET's theory.

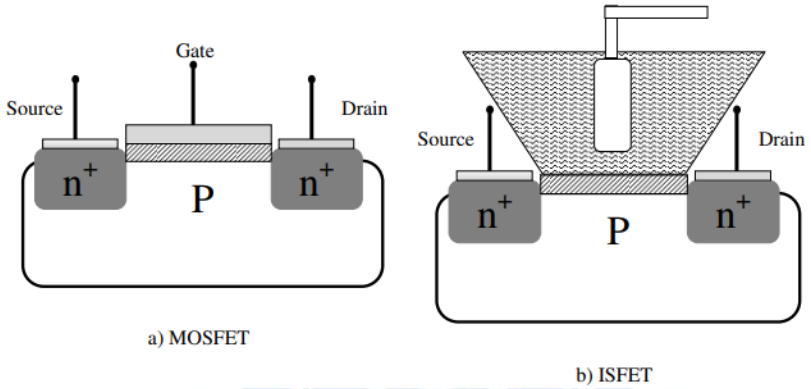
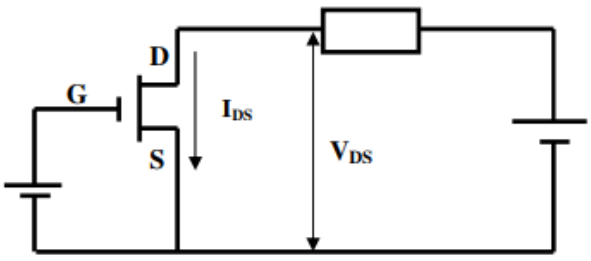


Fig. 5.2.2. *Schematic representation of (a) MOSFET, (b) ISFET*



(c) Electronic diagram

- ISFETs have a structure that is comparable to MOSFETs, and a reference electrode and electrolyte gate replace the metal gate; consequently, the signal transfer mechanism is comparable.

- The sensitive film is applied to the oxide layer to detect other types of ions and molecules. The solution is connected directly with the oxide and achieves linear detection of ions.
- The working of ISFET is explained in the following Steps
  1. **Selective Membrane**: The gate of the transistor is coated with a selective membrane that allows only specific ions to pass through. This membrane can be tailored to be selective to certain ions, such as hydrogen ions (H^+), sodium ions (Na^+), potassium ions (K^+), or others.
  2. **Ion Interaction**: When the solution containing ions comes into contact with the selective membrane, ions in the solution interact with the membrane. Depending on their charge and size, some ions will permeate through the membrane while others will be repelled.
  3. **Change in Potential**: The interaction between the ions and the selective membrane causes a change in the electric potential at the gate of the transistor.
  4. **Transistor Response**: This change in potential alters the conductivity of the transistor channel, which can be measured as a change in current between the source and drain terminals.
  5. **Ion Concentration Measurement**: By measuring the change in current, the concentration of ions in the solution can be determined. The greater the concentration of ions, the larger the change in current.

Applications of Ion selective field effect transistor:

1. **pH Sensing:**
   One of the most common applications of ISFETs is in pH sensing. ISFETs can determine the pH level accurately.
2. **Biomedical Applications**:
   ISFETs are used in various biomedical applications such as biosensors for monitoring glucose levels, detecting biomarkers in bodily fluids, and studying ion channel activity in cells. They are also utilized in the development of implantable devices for continuous monitoring of physiological parameters.
3. **Chemical Analysis**: ISFETs are used in chemical analysis techniques such as ion chromatography and ion-selective electrode potentiometry.

4. Biotechnology and Pharmaceutical Research:

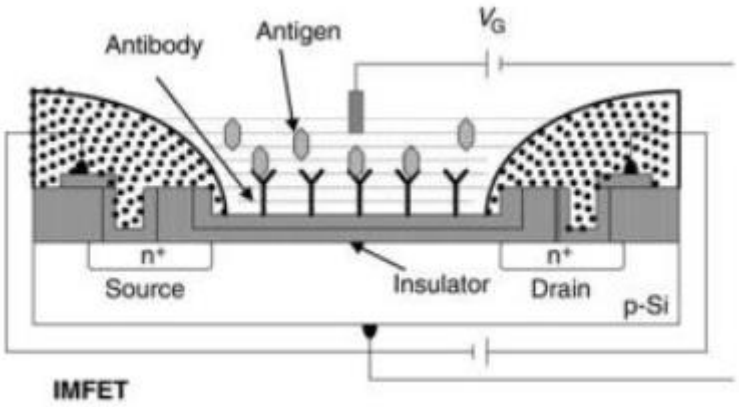
ISFETs play a significant role in biotechnology and pharmaceutical research by enabling the real-time monitoring of biochemical reactions, enzymatic processes, and drug interactions.

5.2.2 Immunologically Sensitive Field Effect Transistor (IMFET):

Structure and Operation of IMFETs:

1. **Field Effect Transistor (FET) Basics**:
    - **Source**: The terminal through which carriers enter the channel.
    - **Drain**: The terminal through which carriers leave the channel.
    - **Gate**: The control terminal that modulates the channel's conductivity.
    - **Substrate**: The body of the transistor, typically made of a semiconductor material.
2. **Immunological Layer**:
    - **Immobilization**: Antibodies or antigens are immobilized on the gate surface of the FET.
    - **Specific Binding**: The immobilized molecules specifically bind to their target antigens or antibodies from the sample.
3. **Detection Mechanism**:
    - **Binding Event**: When the target molecule binds to the immobilized antibody/antigen, it induces a change in the local electric field.
    - **Signal Transduction**: This change in the electric field affects the surface potential and modulates the current flowing through the FET.

- **Measurement**: The change in current or voltage is measured and is proportional to the concentration of the target molecule.



Applications of IMFETs:

1. **Medical Diagnostics**:
   - **Disease Biomarkers**: Detection of specific proteins or antibodies related to diseases such as cancer, HIV, and cardiovascular diseases.
   - **Point-of-Care Testing**: Rapid diagnostics at the patient's side without the need for extensive laboratory equipment.
2. **Environmental Monitoring**:
   - **Pollutants and Toxins**: Detection of hazardous substances such as heavy metals, pesticides, and pathogenic microorganisms in air, water, and soil.
   - **Real-Time Monitoring**: Continuous surveillance of environmental samples for immediate detection of contaminants.
3. **Food Safety**:
   - **Pathogen Detection**: Identifying contamination by bacteria, viruses, and toxins in food products.
   - **Quality Control**: Ensuring the safety and quality of food during production and before consumption.
4. **Biotechnological Research**:
   - **Protein-Protein Interactions**: Studying interactions between various proteins to understand biological pathways.

- **Drug Discovery**: Screening potential drug candidates by detecting their interaction with target biomolecules.

Advantages of IMFETs:

1. **High Sensitivity**:
   - Capable of detecting very low concentrations of target molecules due to the amplification effect of the FET.
2. **Specificity**:
   - Utilizes highly specific antigen-antibody interactions, reducing the likelihood of false positives.
3. **Rapid Response**:
   - Provides real-time monitoring and fast response times, ideal for point-of-care diagnostics.
4. **Miniaturization**:
   - Potential for miniaturization, allowing for the development of portable and compact devices.

Challenges and Considerations:

1. **Stability**:
   - Ensuring the stability of the immobilized biomolecules over time to maintain consistent performance.
2. **Non-Specific Binding**:
   - Minimizing interference from non-specific binding or other environmental factors that could affect accuracy.
3. **Manufacturing Complexity**:
   - Fabricating IMFETs with consistent quality and performance can be challenging and may involve complex processes.

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UNIT- V BIOCHEMICAL MEASUREMENTS

5.3 Blood Glucose Sensors

Accurate measurement of blood glucose is essential in the diagnosis and long-term management of diabetes. This section reviews the use of biosensors for continuous measurement of glucose levels in blood and other body fluids.

Glucose is the main circulating carbohydrate in the body. In normal, fasting individuals, the concentration of glucose in blood is very tightly regulated—usually between 80 and 90 mg/100 ml, during the first hour or so following a meal. The hormone insulin, which is normally produced by beta cells in the pancreas, promotes glucose transport into skeletal muscle and adipose tissue. In those suffering from diabetes mellitus, insulin-regulated uptake is compromised, and blood glucose can reach concentrations ranging from 300 to 700 mg/100 ml (hyperglycemia).

Accurate determination of glucose levels in body fluids, such as blood, urine, and cerebrospinal fluid, is a major aid in diagnosing diabetes and improving the treatment of this disease. Blood glucose levels rise and fall several times a day, so it is difficult to maintain normoglycemia by means of an "open-loop" insulin delivery approach. One solution to this problem would be to "close the loop" by using a self-adapting insulin infusion device with a glucose-controlled biosensor that could continuously sense the need for insulin and dispense it at the correct rate and time. Unfortunately, present-day glucose sensors cannot meet this stringent requirement (Peura and Mendelson, 1984).

Glucose Oxidase Method The glucose oxidase method used in a large number of commercially available simple test strip meters allows quick and easy blood glucose measurements. A test strip product, One Touch UltraMini (www.LifeScan.com), depends on the glucose oxidase–peroxidase chromogenic reaction. After a drop of blood is combined with reagents on the test strip, the reaction shown in (10.18) occurs.

$$\text{Glucose} + 2H_2O + O_2 \xrightarrow{\text{glucose oxidase}} \text{Gluconic Acid} + 2H_2O_2 \qquad (10.18)$$

Adding the enzymes peroxidase and o-dianiside, a chromogenic oxygen, results in the formation of a colored compound that can be evaluated visually.

$$\text{o-dianisine} + H_2O_2 \xrightarrow{\text{peroxidase}} \text{oxidized o-dianisine} + H_2O \qquad (10.19)$$

Glucose oxidase chemistry in conjunction with reflectance photometry produces a system for monitoring blood glucose levels (Burtis and Ashwood, 1994). In the One Touch system (Figure 10.23), a test strip is inserted into the meter, a drop of blood is applied to end of the test strip, and a digital screen displays the results 5 s later.

Electroenzymatic Approach Electroenzymatic sensors based on polarographic principles utilize the phenomenon of glucose oxidation with a glucose oxidase enzyme (Clark and Lyons, 1962). The chemical reaction of glucose with oxygen is catalyzed in the presence of glucose oxidase. This causes a decrease in the partial pressure of oxygen (P_{O_2}), an increase in pH, and the production of hydrogen peroxide by the oxidation of glucose to gluconic acid according to equation (10.18).

Investigators measure changes in all of these chemical components in order to determine the concentration of glucose. The basic glucose enzyme electrode utilizes a glucose oxidase enzyme immobilized on a membrane or a gel matrix, and an oxygen-sensitive polarographic electrode. Changes in oxygen concentration at the electrode, which are due to the catalytic reaction of glucose and oxygen, can be measured either amperometrically or potentiometrically.

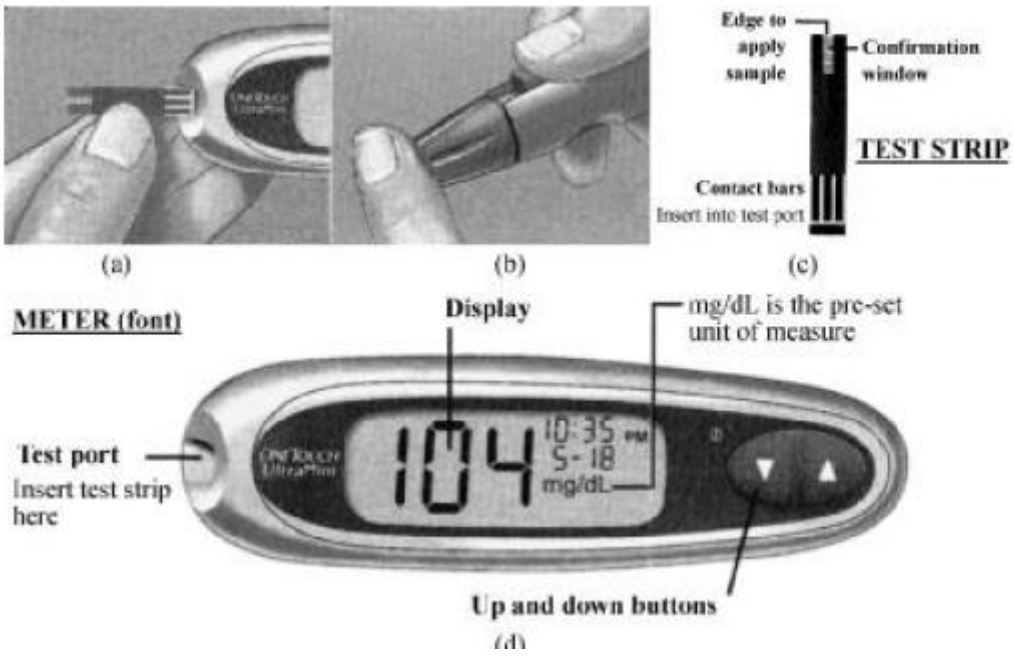
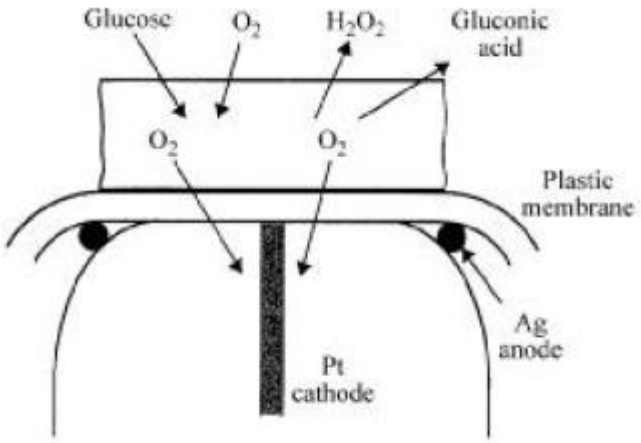


Figure 10.23 (a) A test strip is inserted into the meter. (b) A lance is released to lance the skin less than 1 mm. (c) The 1 μL blood sample is applied to the end of the test strip and drawn into it by capillary action. (d) Then 5 s later, the meter displays the blood glucose in mg/dL.

Because a single-electrode technique is sensitive both to glucose and to the amount of oxygen present in the solution, a modification to remove the oxygen response by using two polarographic oxygen electrodes has been suggested (Updike and Hicks, 1967). Figure 10.24 illustrates both the principle of the enzyme electrode and the dual-cathode enzyme electrode. An active enzyme is placed over the glucose electrode, which senses glucose and oxygen. The other electrode senses only oxygen. The amount of glucose is determined as a function of the difference between the readings of these two electrodes. More recently, development of hydrophobic membranes that are more permeable to oxygen than to glucose has been described (Gilligan *et al.*, 2004). Placing these membranes over a glucose enzyme electrode solves the problem associated with oxygen limitation and increases the linear response of the sensor to glucose.

The major problem with enzymatic glucose sensors is the instability of the immobilized enzyme and the fouling of the membrane surface under physiological conditions. Most glucose sensors operate effectively only for short periods of time. In order to improve the present sensor technologies, more highly selective membranes must be developed. The features that must be taken into account in designing and fabricating these membranes include the diffusion rate of both oxygen and glucose from the external medium to the surface of the membrane, diffusion and concentration gradients within the membrane, immobilization of the enzyme, and the stability of the enzymatic reaction (Jaffari and Turner, 1995).



(a)

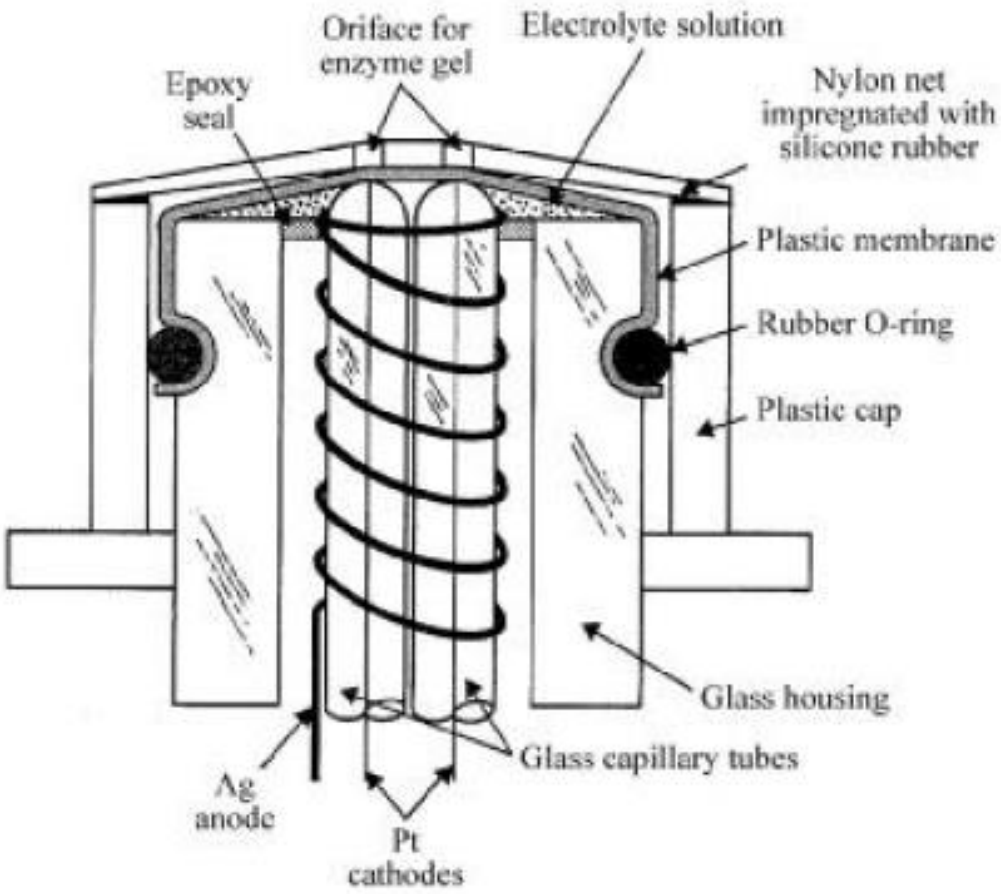


Figure 10.24 (a) In the enzyme electrode, when glucose is present it combines with O_2, so less O_2 arrives at the cathode. (b) In the dual-cathode enzyme electrode, one electrode senses only O_2 and the difference signal measures glucose independent of O_2 fluctuations. (From S. J. Updike and G. P. Hicks, "The enzyme electrode, a miniature chemical transducer using immobilized enzyme activity," *Nature*, 1967, 214, 986–988. Used by permission.)

Optical Approach A number of innovative glucose sensors, based on different optical techniques, has been developed in recent years. A new fluorescence-based affinity sensor has been designed for monitoring various metabolites, especially glucose in the blood plasma (Schultz *et al.*, 1982). The method is similar in principle to that used in radioimmunoassays. It is based on the immobilized competitive binding of a particular metabolite and fluorescein-labeled indicator with receptor sites specific for the measured metabolite and the labeled ligand (the molecule that binds).

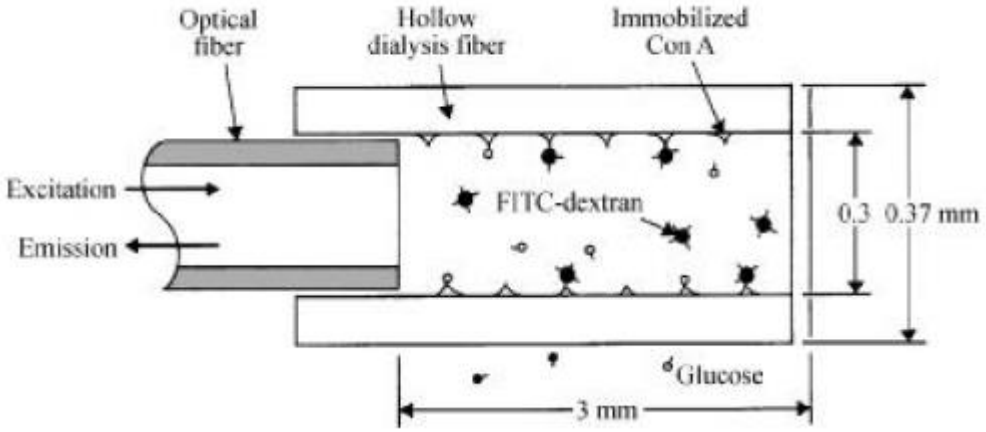


Figure 10.25 The affinity sensor measures glucose concentration by detecting changes in fluorescent light intensity caused by competitive binding of a fluorescein-labeled indicator. (From J. S. Schultz, S. Manouri, *et al.*, "Affinity sensor: A new technique for developing implantable sensors for glucose and other metabolites,"*Diabetes Care*, 1982 5, 245–253. Used by permission.)

Figure 10.25 shows an affinity sensor in which the immobilized reagent is coated on the inner wall of a glucose-permeable hollow fiber fastened to the end of an optical fiber. The fiber-optic catheter is used to detect changes in fluorescent light intensity, which is related to the concentration of glucose. These researches have demonstrated the simplicity of the sensor and the feasibility of its miniaturization, which could lead to an implantable glucose sensor. Figure 10.26 is a schematic diagram of the optical system for the affinity sensor. The advantage of this approach is that it has the potential for miniaturization and for implantation through a needle. In addition, as with other fiber-optic approaches, no electric connections to the body are necessary.

The major problems with this approach are the lack of long-term stability of the reagent, the slow response time of the sensor, and the dependence of the measured light intensity on the amount of reagent, which is usually very small and may change over time.

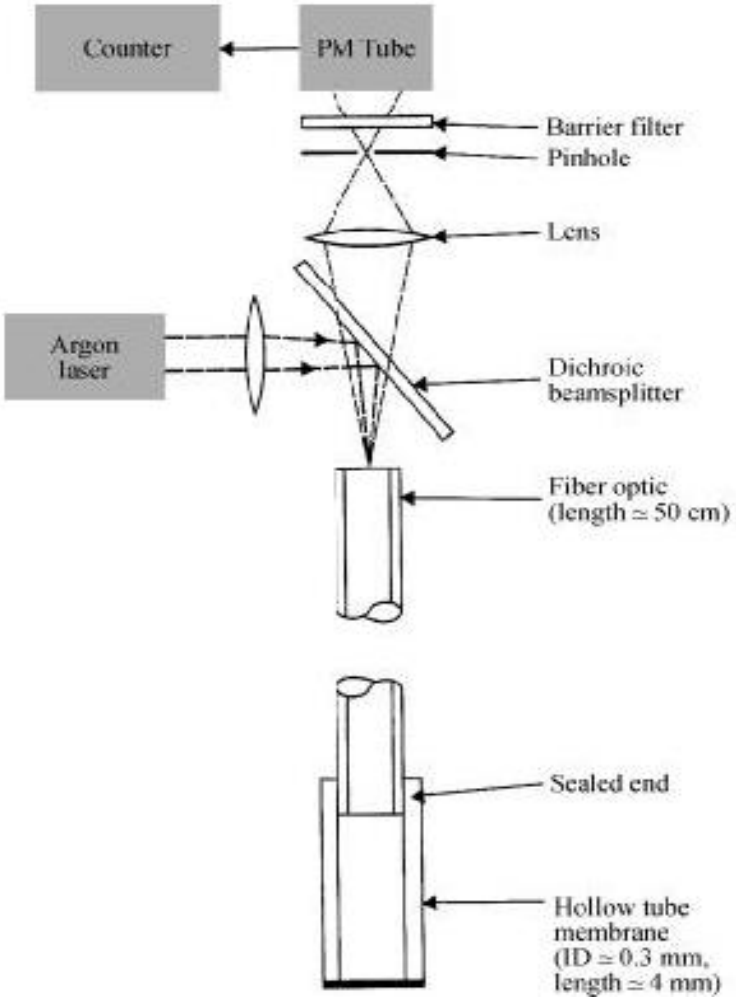


Figure 10.26 The optical system for a glucose affinity sensor uses an argon leaser and a fiber-optic catheter. (From J. S. Schulz, S. Manouri, *et al.*, "Affinity sensor: A new technique for developing implantable sensors for glucose and other metabolites," *Diabetes Care*, 1982, 5, 245–253. Used by permission.)

Attenuated Total Reflection (ATR) and Infrared Absorption Spectroscopy

The application of multiple infrared ATR spectroscopy to biological media is another potentially attractive noninvasive technique. By this means, the infrared spectra of blood can be recorded from tissue independently of the sample thickness, whereas other optical-transmission techniques are strongly dependent on the optical-transmission properties of the medium. Furthermore, employing a laser light source makes possible considerable improvement of the measuring sensitivity. This is of particular interest when one is measuring the transmission of light in aqueous solutions, because it counteracts the intrinsic attenuation of water, which is high in most wavelength ranges.

Absorption spectroscopy in the infrared (IR) region is an important technique for the identification of unknown biological substances in aqueous solutions. Because of vibrational and rotational oscillations of the molecule, each molecule has specific resonance absorption peaks, which are known as *fingerprints*. These spectra are not uniquely identified; rather, the IR absorption peaks of biological molecules often overlap. An example of such a spectrum is shown in Figure 10.27, which is the characteristic IR spectrum of anhydrous D-glucose in the wavelength region 2.5 to 10 μm. The strongest absorption peak, around 9.7 μm, is due to the carbon–oxygen–carbon bond in the molecule's pyran ring.

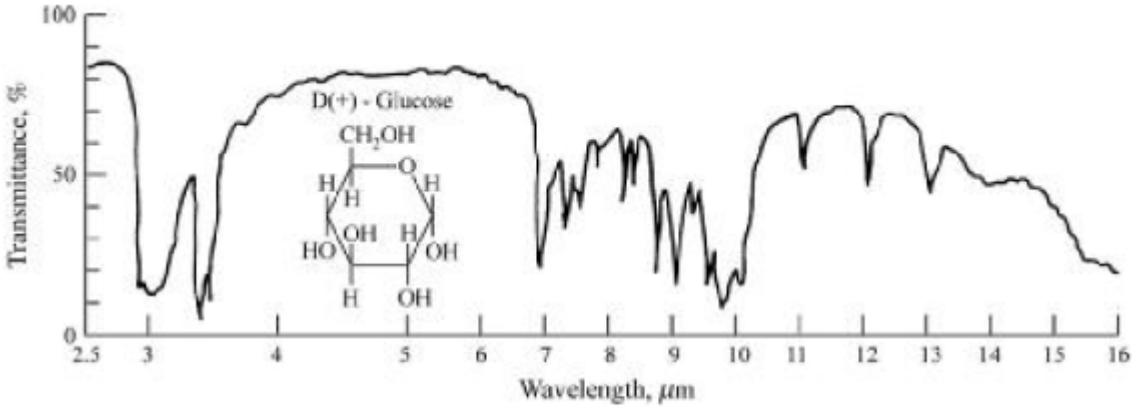


Figure 10.27 The infrared absorption spectrum of anhydrous D-glucose has a strong absorption peak at 9.7 mm. (From Y. M. Mendelson, A. C. Clermont, R. A. Peura, and B. C. Lin, "Blood glucose measurement by multiple attenuated total reflection and infrared absorption spectroscopy," *IEEE Trans. Biomed Eng.*, 1990, 37, 458–465. Used by permission.)

The absorption-peak magnitude is directly related to the glucose concentration in the sample, and its spectral position is within the wavelength range emitted by a CO_2 laser. Thus a CO_2 laser can be used as a source of energy to excite this bond, and the IR absorption intensity at this peak provides, via Beer's law, a quantitative measure of the glucose concentration in a sample.

Two major practical challenges must be overcome in order to measure the concentration of glucose in an aqueous solution, such as blood, by means of conventional IR absorption spectroscopy. (1) Pure water has an intrinsic high background absorption in the IR region, and (2) the normal concentration of glucose and other analytes in human blood is relatively low (for glucose, it is typically 90 to 120 mg/dl, or mg%).

Significant improvements in measuring physiological concentrations of glucose and other blood analytes by conventional IR spectrometers have resulted from the use of high-power sources of light energy at specific active wavelengths. In the case of glucose, the CO_2 laser serves as an appropriate IR source.

Reference : John G. Webster, "Medical Instrumentation Application and Design", 4th edition, Wiley India Pvt Ltd, New Delhi, 2015

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UNIT- V BIOCHEMICAL MEASUREMENTS

5.4 Blood Gas Analyzers

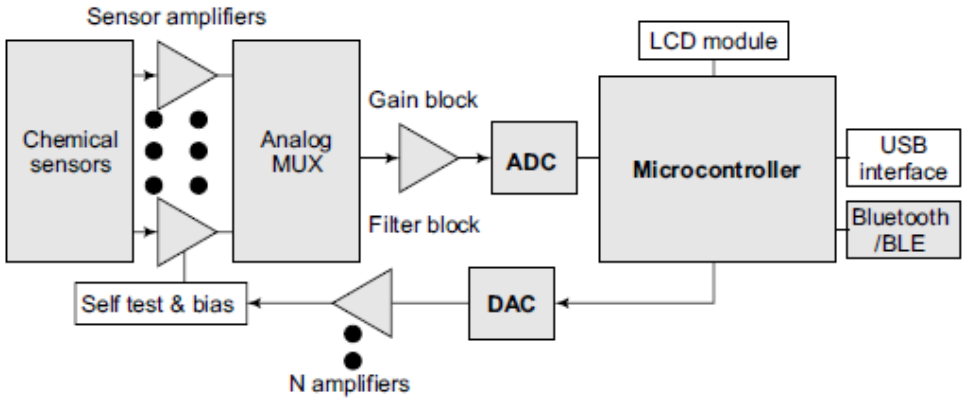
Blood gas analysers are designed to measure pH, pCO2 and pO2 from a single sample of whole blood. The size of the sample may vary from 25 µl to a few hundred microlitres. The estimations take about 1 minute. With built-in calculators, the instruments can also compute total CO2, HCO3 and Base Excess. A typical block diagram of a blood gas analyser machine is shown in Fig. 15.9

In this machine, separate sensors are used for pH, pCO2 and pO2. The outputs from multiple sensors and calculators are driven through a multiplexer to an analog-to-digital converter (ADC). The data is processed in the microcontroller, which is connected to a PC or other instruments through RS-232, USB, or Ethernet. A digital-to-analog converter (DAC) is often used to calibrate the sensor amplifiers to maximize the sensitivity of the electrodes.

Modern blood gas analysers increasingly employ a touch screen in combination with a graphical user interface (GUI) to make the programming process more intuitive. The instrument contains three separate high input impedance amplifiers designed to operate in the specific range of each measuring electrode. A separate module houses and thermostatically controls the three electrodes. It also provides thermostatic control for the humidification of the calibrating gases. A vacuum system provides aspiration and flushing service for all three electrodes. Calibrating gases are selected by a special push button control and passed through the sample chamber when required.

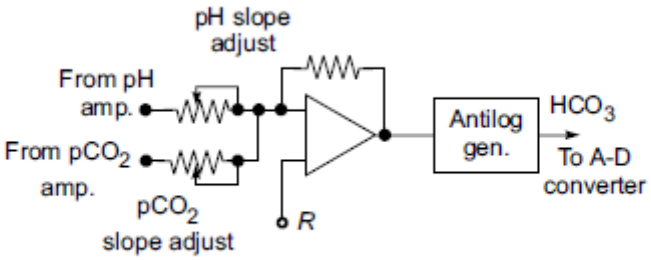
Two gases of accurately known O2 and CO2 percentages are required for calibrating the analyser in the pO2 and pCO2 modes.

The gases required are: O2 value of 12% Cal and 0% Slope and CO2 value of 5% Cal and 10% Slope. These gases are used with precision regulators for flow and pressure control. Two standard buffers of known pH are required for calibration of the analyser in the pH mode. The buffers that are used are 6.838 (Cal) and 7.382 (Slope). It is generally recommended that the sample chamber should control 7.382 buffer when in the standby mode. Microcontroller



➤ Fig. 15.9 *Block diagram of a complete blood gas analyser (Adapted from M/s Texas Instruments)*

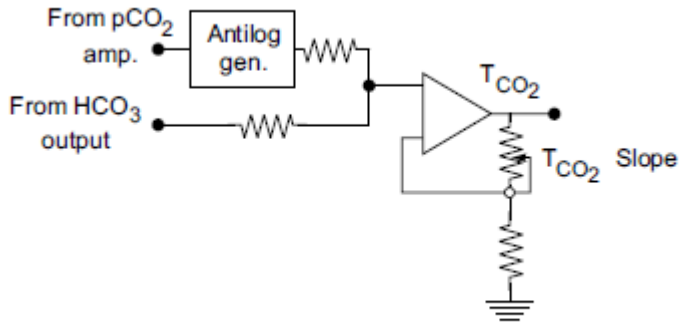
Input signal to the (HCO3) calculator (Fig. 15.10) comes from the outputs of the pH and pCO2 amplifiers. The outputs are suitably adjusted by multiplying each signal by a constant and are given to an adder. The next stage is an antilog-generator similar to the one used in a pCO2 amplifier. The output of this circuit goes to an A–D converter for display. Resistance R is used to adjust zero at the output.



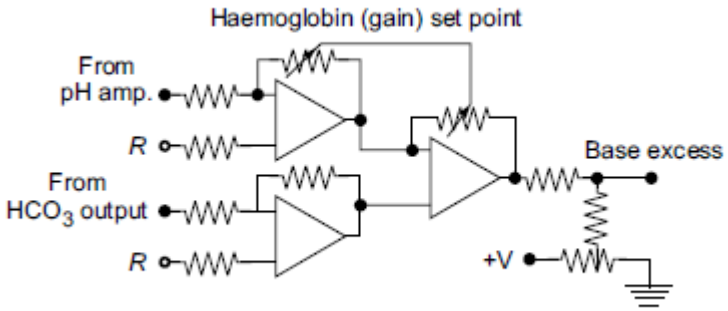
➤ Fig. 15.10 *Circuit diagram for computation of bicarbonate (HCO_3)*

Total CO2 is calculated (Fig. 15.11) by summing the output signals of the (HCO3) calculator and the output of the pCO2 amplifier. Facilities for adjusting the slope and zero at the output are available.

The base excess calculator (Fig. 15.12) consists of three stages. In the first stage, the output of the pH amplifier is inverted in an operational amplifier whose gain is controlled with a potentiometer (Haemoglobin value) placed on the front panel. The output of the HCO– 3 calculator is inverted in the second stage. The third stage is a summing amplifier A3 whose output is given to an A–D converter.



➤ Fig. 15.11 *Circuit diagram for computation of total CO_2*



➤ Fig. 15.12 *Circuit diagram for computation of base excess*

The three electrodes (pH, pO2 and pCO2) are housed in a thermostatically controlled chamber. It also provides thermostatic control for the humidification of the calibrating gases. The thermal block and the humidifier block heat control circuits are of the same type (Fig. 15.13).

The temperature is set with a potentiometer for exactly 37°C. The heater circuit is controlled by a thermistor in the block, which acts as a sensor. As the heat increases, the resistance of the thermistor decreases. At 37°C, the thermistor is calibrated to have a resistance of 25 K ohm. Supposing the temperature of the block decreases, the resistance of the thermistor will increase. The increase in resistance

will cause the voltage at inverting input of op-amp to become more negative. This results in the output voltage becoming more positive, increasing the base current of transistors T1 and T2. The increase in base current increases the collector current, which goes directly to the heater resistor on the block. As the heater resistor heats up the block, the thermistor will decrease until it returns to 25 k ohm.

Many of the blood gas analysers have a provision for checking the membrane of pO2 and pCO2 electrodes. In the check position, a potential is applied across the membrane. Any leak in the membrane of sufficient magnitude will result in a considerable lowering of the resistance may be from 100 MW to 500 kW. The change in resistance can be used to have a change of potential to switch on a transistor, which would cause a lamp to light on the front panel of the instrument. This would indicate that a new membrane is needed.

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5.5 Colorimeter

A colorimeter is an analytical device used to determine the concentration of a colored compound in a solution by measuring the absorbance of a specific wavelength of light.

A basic colorimeter schematic is shown in Figure 5.5.1. Observe that light passes through an optical color filter, is focused by lenses on the reference and sample cuvettes, and falls on the reference and sample photodetectors. The difference in voltage between the two detectors is increased by a dc amplifier and applied to a meter.

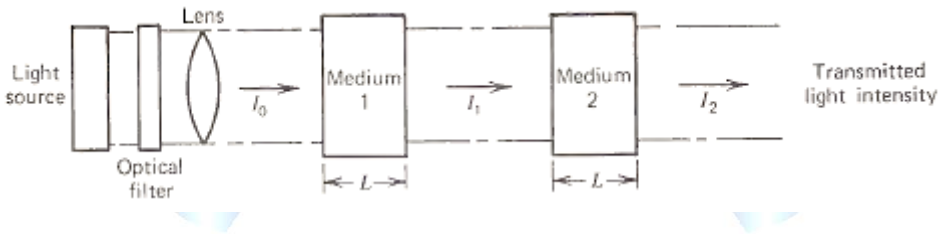

Fig 5.5.1 *Basic colorimeter analysis*

Basic colorimeter analysis (Figure 5.5.1) involves the precise measurement of light intensity. Transmittance is defined as:

$$T = \frac{I_1}{I_0} x100\ \%$$

$$I_2 = TI_1$$

$$I_2 = T^2 I_o$$

Io is initial light intensity

I_1 is first attenuated light intensity

I_2 is second attenuated light intensity

T is transmittance in percent

Absorbance (optical density) is defined as:

$$A = \log\frac{I_1}{I_0} = \log\frac{I}{T}$$

Where,

A is absorbance

If the path length or concentration increases, the transmittance decreases and the absorbance increases. Essentially, this phenomenon can be expressed by Beer's law: The operation of the colorimeter is based on ***Beer-Lambert's law*** which states that the amount of light absorbed by a color solution is directly proportional to the solution's concentration and the length of a light path through it.

A =aCL

where

A is absorbance

L is cuvette path length

C is concentration of absorbing substance

a is absorbtivity related to the nature of the absorbing substance and optical wavelength (known for a standard solution concentration)

Therefore, the concentration of the unknown solution can be found from the following relationship:

$$C_\mu = C_s \frac{A_u}{A_s}$$

where

C_u is unknown concentration

C_s is standard concentration (for calibration)

A_u is unknown absorbance

A_s is standard absorbance

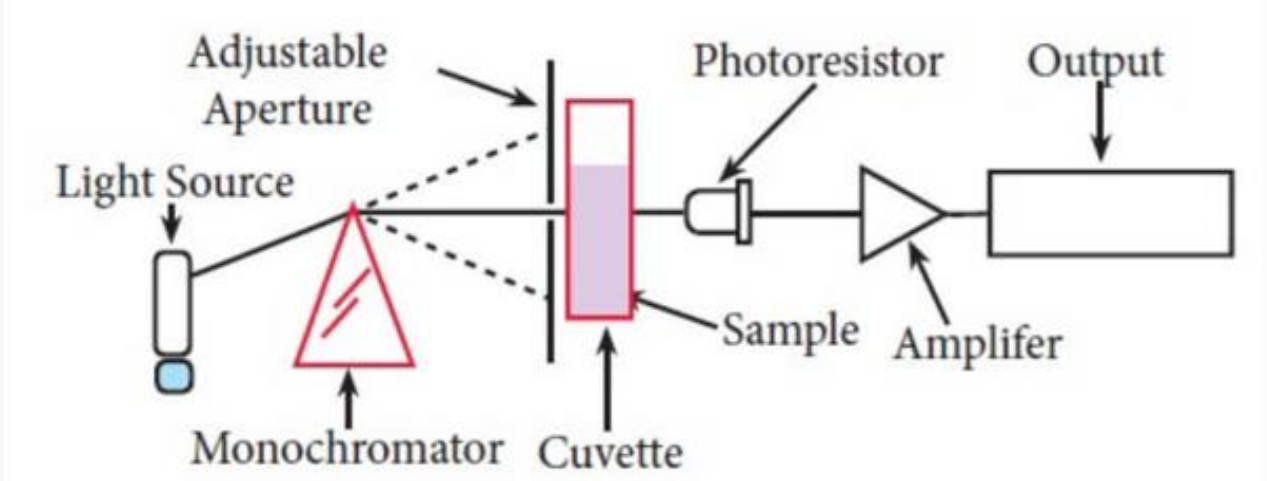

Fig 5.5.2 *Principle of Colorimeter*

Components of a Colorimeter:

1. **Light Source:** The source of light should produce energy with enough intensity to cover the entire visible spectrum (380-780 nm). Commonly, Tungsten lamps are used as a light source for measurement in the visible spectrum and near-infrared ranges. Halogen deuterium is suitable for measurement in the UV range (200-900 nm).
2. **Slit:** It reduces unwanted or stray light by allowing a light beam to pass through.
3. **Condensing lens:** Parallel beam of light emerges from condensing lens after the light passes through slit incidents on it.
4. **Monochromator:** It filters the monochromatic light from polychromatic light, which absorbs unwanted light wavelengths and permits only monochromatic light. These are of three types: prism, grating, and glass.
    a. **Prism:** It facilitates the refraction of light when it passes from one medium to another.
    b. **Glass:** It selectively transmits light in certain ranges of wavelengths.
    c. **Gratings:** These are made of graphite, which separates light in different wavelengths.

5. **Cuvette (Sample cell):** The monochromatic light from the filter passes through the colored sample solution placed in the cuvette. Their sizes range from square, and rectangle to round and have a fixed diameter of 1cm. These are of three types based on the substances these are made of: Glass, Quartz, and Plastic cuvette.
    a. **Glass cuvettes** are cheap and absorb light of 340 nm wavelength.
    b. **Quartz cuvettes** facilitate entry of both lights of UV and visible ranges.
    c. **Plastic cuvettes** are cheaper, easily scratched, and have shorter lifespans.
6. **Photocell (Photodetector**): These photosensitive devices measure light intensity by converting light energy into electrical energy.
7. **Galvanometer:** The electrical signal generated in a photocell is detected and measured by a galvanometer. It displays optical density (OD) and percentage transmission.

Working Principle:

1. **Light Emission**: The light source emits a beam of light that passes through the monochromator or filter. This component isolates the specific wavelength of light that corresponds to the maximum absorbance of the substance being measured.
2. **Light Transmission Through Sample**: The selected wavelength of light passes through the sample solution in the cuvette. If the sample contains a colored compound, it will absorb some portion of this light. The amount of light absorbed is proportional to the concentration of the colored compound in the solution.
3. **Detection**: The light that has not been absorbed passes through the sample and reaches the photodetector. The photodetector converts this light into an electrical signal.
4. **Measurement**: The electrical signal is processed and converted into a readable value, usually displayed as absorbance (A) or transmittance (%T).

Applications of Colorimeter:

- These are used in the food and ***food processing*** industries.
- It is frequently used in laboratories and hospitals to determine the ***biochemical composition*** of samples like blood, urine, cerebral spinal fluid, plasma, serum, etc.
- The instrument is also employed in cosmetology to measure the UV protection level of ***skin-care products***.
- They are used to evaluate the ***water's purity*** and screen for the presence of chemicals like cyanide, iron, fluorine, chlorine, molybdenum, etc.
- They are employed to evaluate the color contrast and brightness of ***screens on mobile devices***, computers, and televisions to give people the greatest viewing experience.
- A colorimeter is also employed in the ***pharmaceutical*** sector.
- Blood samples are tested using a colorimeter to determine the amount of ***hemoglobin*** present.

Advantages of Colorimeter:

1. User-friendly interfaces and minimal calibration steps make them accessible for routine analysis.
2. Colorimeters provide quick result.
3. Many colorimeters are portable and can be used in field settings, allowing for on-site analysis.

Disadvantages of Colorimeter:

1. Colorimeters generally have lower sensitivity and precision compared to spectrophotometers.
2. Colorimeters typically measure absorbance at a limited number of fixed wavelengths.
3. Colorimetric measurements can be affected by the presence of other colored substances or turbidity in the sample.
4. Accurate measurements depend on proper calibration using standard solutions.

5. Colorimeters are generally designed to measure one parameter at a time, unlike more advanced instruments that can analyze multiple parameters simultaneously.

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UNIT- V BIOCHEMICAL MEASUREMENTS

5.6 Flame Photometer

- The flame photometer, shown in Figure 5.6.1 measures the color intensity of a flame that is supported by oxygen and a specific substance.
- The flame photometer is one of the most useful instruments in clinical analyses.
- The method of flame photometric determinations is simple.
- A flame photometer is an analytical instrument used to measure the concentration of certain metal ions, such as sodium (Na), potassium (K), calcium (Ca), and lithium (Li), in a sample.
- It works on the principle of flame emission spectroscopy, where the intensity of the light emitted by the flame is measured to determine the concentration of the metal ions.

Principle of Flame photometer:

1. A solution of the sample to be analyzed is prepared. A special sprayer operated by compressed air or oxygen is used to introduce this solution in the form of a fine spray (aerosol) into the flame of a burner operating on some fuel gas, like acetylene or hydrogen.
2. The radiation of the element produced in the flame is separated from the emission of other elements by means of light filters or a monochromator.
3. The intensity of the isolated radiation is measured from the current it produces when it falls on a photodetector.
4. The measurement of current is done with the help of a readout meter, whose readings are proportional to the concentration of the element. After carefully calibrating the meter with solutions of known composition and concentration, it

is possible to correlate the intensity of a given spectral line of the unknown sample, with the amount of the same element present in a standard solution.

Construction of Flame Photometer:

A flame photometer has three essential parts (Fig 5.6.1). These are:

(a) Emission System: consists of the following:

(i) Fuel gases: and their regulation: comprising the fuel reservoir, compressors, pressure regulators and pressure gauges.

(ii) Atomizer: consisting, in turn, of the sprayer and the atomization chamber, where the aerosol is produced and fed into the flame.

(iii) Burner: receives the mixture of the combustion gases.

(iv) Flame: the true source of emission.

(b) ***Optical System***: It consists of the optical system for wavelength selection (filters or monochromators), lenses, diaphragms, slits etc.

(c) ***Recording System***: It includes detectors like photocells, photo-tubes, photomultipliers, photodiodes etc. and the electronic means of amplification, measuring and recording.

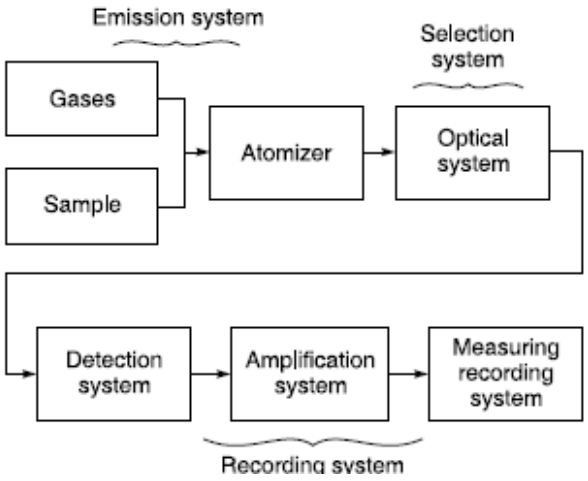

Fig. 5.6.1 *Essential parts of a flame photometer*

Dedicated instruments for the simultaneous analysis of sodium, potassium and lithium are available for clinical applications. In these instruments, sample handling is automatic, as the system has a turntable, which will hold up to 20 samples in cups and an automatic positive piston displacement dilutor, that dilutes the sample prior to entering the spray chamber.

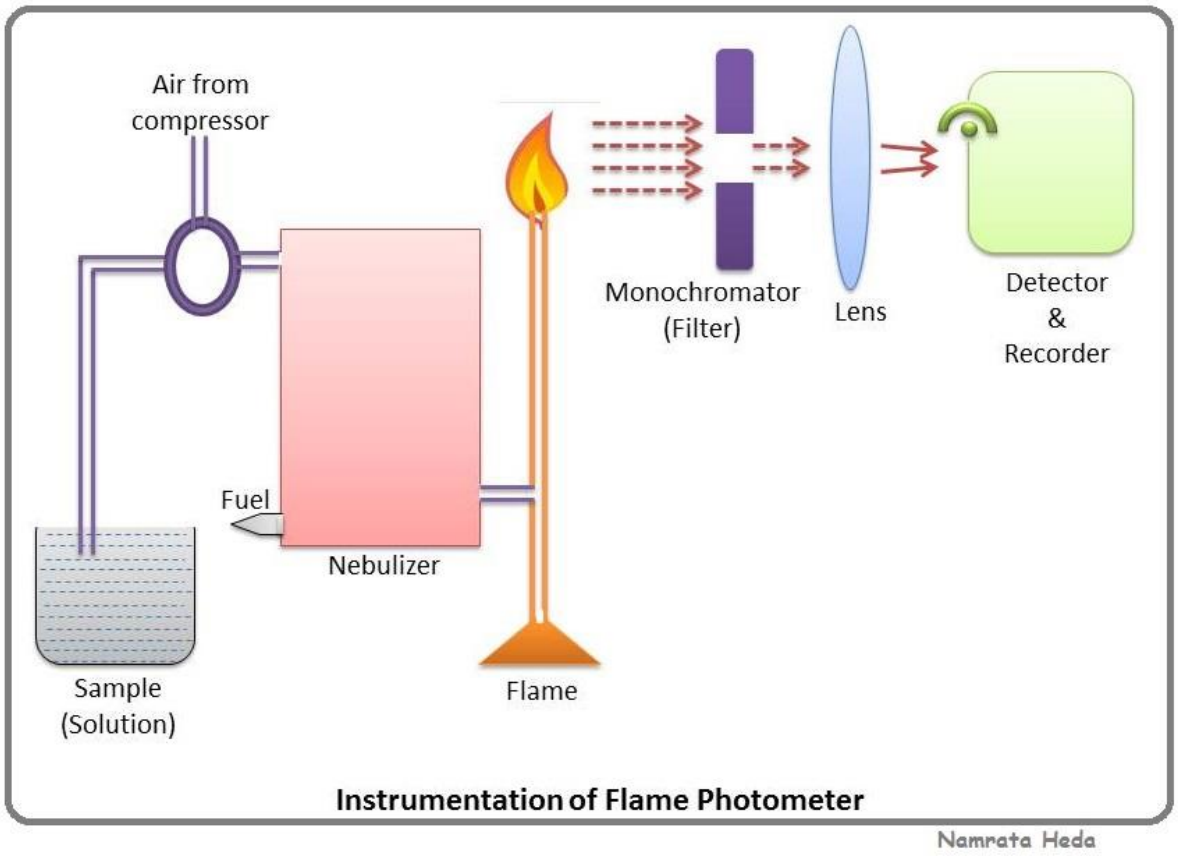

Fig. 5.6.2 Flame Photometer

Working of Flame Photometer:

- **Sample Preparation:**

  The sample is prepared by diluting it with a suitable solvent, often distilled water, to bring the concentration of metal ions within the measurable range of the flame photometer.

- **Sample Aspiration:**

  The prepared sample is aspirated into the nebulizer, where it is converted into a fine mist and introduced into the flame.

- **Excitation:**

  In the flame, the metal ions are excited to higher energy levels. As they return to their ground state, they emit light at characteristic wavelengths specific to each metal ion.

- **Wavelength Isolation:**

  The emitted light is passed through the monochromator, which isolates the characteristic wavelength of the metal ion being measured.

- **Detection and Measurement:**

  The isolated light is detected by the photodetector, which generates an electrical signal proportional to the intensity of the light.

- **Data Processing:**

  The electrical signal is processed and converted into a concentration reading, which is displayed on the readout device.

Calibration :

Calibration is a critical part of flame photometry. The instrument is calibrated using standard solutions of known concentrations of the metal ion of interest. A calibration curve is plotted by measuring the intensity of light emitted at different concentrations. This curve is then used to determine the concentration of metal ions in unknown samples.

Applications :

Flame photometers are widely used in various fields, including:

- ✓ **Clinical Laboratories:**
  - For measuring electrolytes such as sodium, potassium, and calcium in biological fluids.
- ✓ **Agriculture:**
  - For soil and plant analysis to determine nutrient levels.
- ✓ **Environmental Monitoring:**
  - For analyzing water samples for metal contamination.
- ✓ **Food Industry:**
  - For quality control by measuring mineral content in food products.

Advantages of flame photometer

i. The method of analysis is ***very simple*** and economical.
ii. It is quick, convenient, selective and ***sensitive analysis***.
iii. It is both ***qualitative*** and ***quantitative*** in nature.
iv. Even very ***low concentrations*** (parts per million/ppm to parts per billion/ppb range) of metals in the sample can be determined.
v. This method compensates for any ***unexpected interfering material*** present in the sample solution.
vi. This method can be used to ***estimate elements*** which are rarely analysed.

Disadvantages of flame photometer:

In spite of many advantages, this analysis technique has quite a few disadvantages:

i. The ***accurate concentration*** of the metal ion in the solution ***cannot*** be measured.
ii. It cannot directly detect and determine the ***presence of inert gases***.
iii. Though this technique measures the total metal content present in the sample, it does ***not provide*** the information about the ***molecular structure*** of the metal present in the sample.
iv. ***Only liquid samples*** may be used. Also sample preparation becomes lengthy in some cases

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5.7 Spectrophotometer

A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. It is commonly used in chemistry, physics, biology, and industrial applications to quantify the concentration of substances in a solution by measuring the amount of light absorbed by the solution.

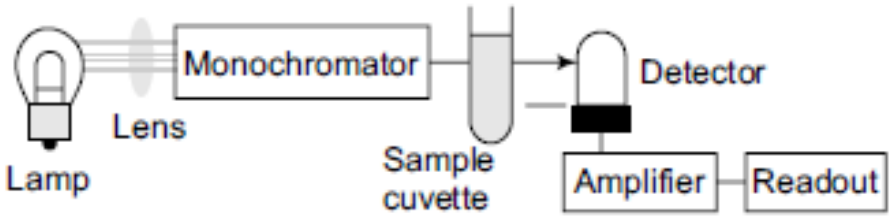


Fig. 5.7.1 *Various components of a spectrophotometer type instrument*

Fig. 5.7.1 shows the various components used in a spectrophotometer.

Basic Components of a Spectrophotometer

1. **Light Source**:
   - ❒ Provides the initial light that will pass through the sample.
   - ❒ Common light sources include tungsten lamps for visible light and deuterium lamps for ultraviolet light.

2. **Monochromator**: ***(Filtering arrangement)***

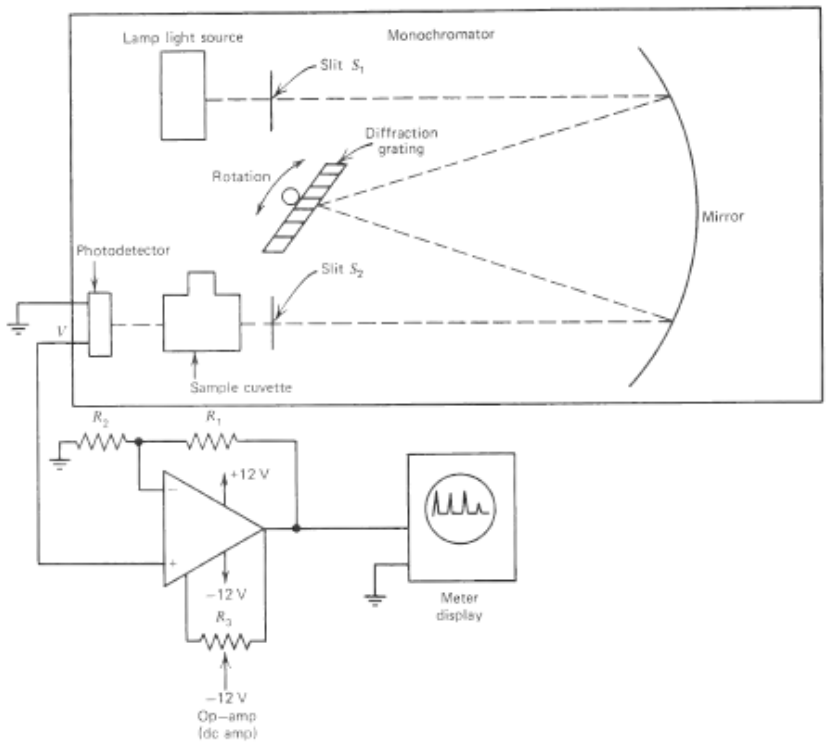
- ❒ Separates the light into its component wavelengths (i.e., it selects a specific wavelength of light).
- ❒ Can be a prism or diffraction grating.

3. **Optical System**:
   - ❒ An Optical system produces a parallel beam of filtered light for passage through an absorption cell / sample holder (cuvette).
   - ❒ This may include lens, mirrors, slits, diaphragms etc.

4. **Sample Holder**:
   - ❒ Contains the sample being analyzed.
   - ❒ Often a cuvette, which is a small, transparent container.

5. **Detector**:
   - ❒ Measures the intensity of light passing through the sample (unabsorbed radiant energy)
   - ❒ Common detectors include human eye, a barrier-layer cell, phototube, photo-multiplier tube or photodiodes.

6. **Digital Display or Output Device**:
   - ❒ Shows the results of the measurement.
   - ❒ Can be a digital readout or a connected computer.



***5.7.2** Spectrophotometer—simplified schematic*

5.7.1 Radiation Sources:

- The function of the radiation source is to provide a sufficient intensity source of light which is suitable for making a measurement.
- The most common and convenient source of light is the ***tungsten lamp***.
- This lamp consists of a ***tungsten filament*** enclosed in a glass envelope.
- It is cheap, intense and reliable.
- A major portion of the energy emitted by a tungsten lamp is in the visible region and only about 15 to 20% is in the infrared region.
- When using a tungsten lamp, it is desirable to use a ***heat absorbing filter*** between the lamp and the sample holder to absorb most of the infrared radiation.
- For work in the ultraviolet region, a ***hydrogen or deuterium*** discharge lamp is used.
- For work in the infrared region, a tungsten lamp may be used. However, due to high absorption of the glass envelope and the presence of unwanted emission in the visible range, tungsten lamps are not preferred. In such cases, nernst filaments or other sources of similar type are preferred.

- Modern instruments use a ***tungsten-halogen*** light source, which has a higher intensity output than the normal tungsten lamp.

5.7.2 Optical Filters:

- Ideal filters should be monochromatic, i.e. they must isolate radiation of only one wavelength. A filter must meet the following two requirements:
  - ✓ high transmittance at the desired wavelength and
  - ✓ low transmittance at other wavelengths

- **Absorption Filters**:
  a) The absorption type optical filter usually consists of colour media: colour glasses, coloured films (gelatin, etc.), and solutions of the coloured substances.
  b) This type of filter has a wide spectral bandwidth. Their efficiency of transmission is very poor and is of the order of 5 to 25%.

- **Interference Filters**:
  1. These filters usually consist of two semi-transparent layers of silver, deposited on glass by evaporation in vacuum and separated by a layer of dielectric (ZnS or MgF2).
  2. Interference filters allow a much narrower band of wavelengths to pass and are similar to monochromators in selectivity.
  3. The transmittance of these filters varies between 15 to 60per cent with a spectral bandwidth of 10 to 15 nm.
  4. For efficient transmission, multilayer transmission filters are often used.
  5. They are characterized by a bandpass width of 8 nm or less and a peak transmittance of 60-95%.

5.7.3 Monochromators:

1. Monochromators are optical systems, which provide better isolation of spectral energy than the optical filters, and are therefore preferred where it is required to isolate narrow bands of radiant energy.

2. Monochromators usually incorporate a small glass of quartz prism or a diffraction grating system as the dispersing media.
3. The radiation from a light source is passed either directly or by means of a lens or mirror into the narrow slit of the monochromator and allowed to fall on the dispersing medium, where it gets isolated

❒ **Prism Monochromators**:

i. Prism may be made of glass or quartz. The glass prisms are suitable for radiations essentially in the visible range whereas the quartz prism can cover the ultraviolet spectrum also.

ii. Prism spectrometers are usually expensive, because of exacting requirements and difficulty in getting quartz of suitable dimensions.

❒ **Diffraction Gratings**:

i. Monochromators may also make use of diffraction gratings as a dispersing medium.

ii. A diffraction grating consists of a series of parallel grooves ruled on a highly polished reflecting surface.

iii. When the grating is put into a parallel radiation beam, so that one surface of the grating is illuminated, this surface acts as a very narrow mirror.

iv. The reflected radiation from this grooved mirror overlaps the radiation from neighbouring grooves.

❒ **Holographic Gratings**:

i. Precision spectrophotometers use holographic or interference gratings, which have superior performance in reducing stray light as compared to diffraction gratings.

ii. Holographic gratings are made by first coating a glass substrate with a layer of photo-resist, which is then exposed to interference fringes generated by the intersection of two collimated beams of laser light.

5.7.4 Detectors:

❒ The radiation is fall on a photosensitive element, in which the light energy is converted into electrical energy.

- The electric current produced by this element can be measured with a sensitive galvanometer directly or after suitable amplification.
- Any type of photosensitive detector may be used for the detection and measurement of radiant energy, provided it has a linear response in the spectral band of interest and has a sensitivity good enough for the particular application.
- There are two types of photo-electric cells; photo-voltaic cells and photo-emissive cells.
- Modern spectrophotometers use semiconductor array detectors which allow rapid recording of absorption spectra.
- These spectrometers use photodiode arrays (PDAs) or charge-coupled devices (CCDs) as the detector.

5.7.5 Sample Holders:

- Liquids may be contained in a cell or cuvette made of transparent material such as silica, glass or perspex.
- The faces of these cells through which the radiation passes are highly polished to keep reflection and scatter losses to a minimum.
- Solid samples are generally unsuitable for direct spectrophotometry.
- It is usual to dissolve the solid in a transparent liquid.
- Gases may be contained in cells which are sealed or stoppered to make them air-tight

Working Principle :

1. **Emission of Light**:
    - The light source emits a broad spectrum of light.
2. **Selection of Wavelength**:
    - The monochromator isolates a specific wavelength from the emitted light.
    - This selected wavelength is directed towards the sample.
3. **Light Passage Through Sample**:
    - The light passes through the sample held in the cuvette.

- The sample absorbs some of the light, and the remaining light passes through.

4. **Detection of Transmitted Light**:
   - The detector measures the intensity of the transmitted light (It).
5. **Calculation of Absorbance**:
   - The instrument calculates absorbance (A) using the formula:
   - $$A = -\log\left(\frac{I_t}{I_0}\right)$$

     Where I_t is the intensity of transmitted light and
     I_0 is the intensity of the incident light.
6. **Display of Results**:
   - The absorbance value is displayed on the digital screen or sent to a connected computer for analysis.

Applications

1. **Quantitative Analysis**:
   - Determines the concentration of substances in a solution using Beer-Lambert Law:

     $$A = \epsilon c l$$

     where A is absorbance,
     ϵ is the molar absorptivity,
     c is the concentration, and
     l is the path length of the cuvette.
2. **Kinetic Studies**:
   - Monitors reaction rates by measuring changes in absorbance over time.
3. **Nucleic Acid and Protein Analysis**:
   - Measures concentrations and purity of DNA, RNA, and proteins by detecting specific absorbance wavelengths (e.g., 260 nm for nucleic acids, 280 nm for proteins).
   -

4. **Colorimetric Assays**:
    - Used in assays where a color change indicates the presence or concentration of a substance.

Calibration and Maintenance :

- **Calibration**: Regular calibration with standard solutions is necessary to ensure accurate measurements.
- **Maintenance**: Includes cleaning the light source, monochromator, and sample holder to prevent contamination and maintain precision.

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DEPARTMENT OF BIOMEDICAL ENGINEERING

BM3491 Biomedical Instrumentation

UNIT- V BIOCHEMICAL MEASUREMENTS

5.8 Blood Cell Counter

5.8.1 Types of Blood Cells:

- The blood constitutes 5–10% of the total body weight and in an average adult, it amounts to 5–6 litres.
- Blood consists of corpuscles suspended in a fluid called plasma in the proportion of 45 parts of corpuscles (cells) to 55 parts of plasma.
- The percentage of cells in the blood is called the haematocrit value or packed cell volume (PCV).
- The majority of the corpuscles in blood are red blood cells (erythrocytes), others being white blood cells (leucocytes) and platelets (thrombocytes).

Erythrocytes (Red Blood Cells):

1. Red blood cells have the form of a bi-concave disc with a mean diameter of about 7.5 μ and thickness of about 1.7 μ. The mean surface area of the cell is about 134 μm^2.
2. There are about 5.5 million of them in every cubic millimetre of blood in men and nearly 5 million in women.
3. In the whole body, there are about 25 billion erythrocytes and they are constantly being destroyed and replaced at a rate of about 9000 million per hour.
4. The erythrocytes have no nucleus. They are responsible for carrying oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs.

Leucocytes (White Blood Cells):

1. Leucocytes are spherical cells having a nucleus. There are normally 5000–10,000 white cells per cubic mm of blood but their number varies during the day.
2. They live for seven to fourteen days and there is a rapid turnover, with constant destruction and replacement. Leucocytes form the defence mechanism of the body against infection.
3. They are of two main types: the neutrophils and the lymphocytes.
4. Neutrophils ingest bacteria and lymphocytes are concerned with immunological response.
5. The number and proportion of these types of leucocytes may vary widely in response to various disease conditions.

Thrombocytes (Platelets):

1. Platelets are usually tiny, round, oblong or irregularly shaped cells of the blood with an average diameter of approximately 2 μ.
2. They play an important role in the blood coagulation process.
3. There are usually 250,000–750,000 platelets in every cubic mm of blood.

Calculation of Size of Cells:

1. **Mean Cell Volume (MCV)** is a measure of the average volume of a red blood cell. It is an important parameter in the complete blood count (CBC) and helps in the classification of different types of anemia. MCV is calculated by dividing the total volume of red blood cells by the total number of red blood cells in a given volume of blood.

$$\text{MCV} = \frac{Hematocrit\ (\%)}{Red\ Blood\ Cell\ Count\ (million\ cells/\mu L)}\ X10$$

Normal Range: Adults: 80-100 femtoliters (fL)

2. Mean Cell Haemoglobin (MCH):

Mean Cell Hemoglobin (MCH) is a measure of the average amount of hemoglobin per red blood cell. It is one of the red blood cell indices included in a complete blood count (CBC) and helps in the diagnosis and classification of anemia and other hematological conditions.

$$\text{MCH} = \frac{Hemoglobin\ (g/dL)}{Red\ Blood\ Cell\ Count\ (million\ cells/\mu L)}\ X10$$

Normal Range: Adults: 27-33 picograms (pg) per cell

3. Mean Platelet Volume (MPV):

Mean Platelet Volume (MPV) is a measure of the average size of platelets in the blood. It is included in a complete blood count (CBC) and provides information about platelet production in the bone marrow and platelet activation.

Normal Range: Adults: 7.5-11.5 femtoliters (fL)

METHODS OF CELL COUNTING

- ✓ Microscopic Method
- ✓ Automatic Optical Method
- ✓ Electrical Conductivity Method

Microscopic Method :

1. The most common and routinely applied method of counting blood cells even today, particularly in small laboratories, is the microscopic method in which the diluted sample is visually examined and the cells counted.
2. Commonly known as the counting chamber technique, it suffers from several common drawbacks.
3. Apart from the inherent error of the system, which may be about 10%, there is an additional subjective error of ±10% entailing poor reproducibility of the

results. Furthermore, the lengthy procedure involved results in the rapid tiring of the person making the examination.

4. Another problem with microscopic counting is that the data gathered by this measurement is not directly suitable for storage or for further processing and evaluation.

Procedure:

1. **Preparation of the Blood Sample**

1. **Collect the Blood Sample:** Draw a blood sample and place it in an EDTA tube to prevent clotting.
2. **Mix the Sample:** Gently invert the tube several times to mix the blood with the anticoagulant thoroughly.

2. **Dilution of the Blood Sample**

1. **Prepare Diluting Fluid:** Choose an appropriate diluting fluid based on the type of cells you are counting.
2. **Dilute the Blood:** Using a pipette, draw a small amount of blood and mix it with the diluting fluid. The dilution ratio depends on the type of cells:
    - For RBCs: Typically, a 1:200 dilution.
    - For WBCs: Typically, a 1:20 dilution.

3. **Loading the Hemocytometer**

1. **Clean the Hemocytometer:** Wipe the hemocytometer and cover slip with disinfectant wipes to remove any dust or contaminants.
2. **Charge the Hemocytometer:** Place the cover slip on the hemocytometer. Using a pipette, fill the counting chamber with the diluted blood sample. Allow the sample to spread evenly under the cover slip by capillary action.
3. **Let it Settle:** Allow the cells to settle for a few minutes to ensure an even distribution in the chamber.

4. Counting the Cells

1. **Microscope Setup:** Place the hemocytometer on the microscope stage and focus on the grid lines using low power (10x objective).
2. **Switch to High Power:** Once focused, switch to high power (40x objective) for detailed counting.
3. **Identify the Grid:** Identify the central grid area for RBC counting and the four large corner squares for WBC counting.

5. Counting Procedure

1. **Count Red Blood Cells (RBCs):**
   - Focus on the central large square (divided into 25 smaller squares).
   - Count the cells in five of the smaller squares (four corners and one center).
   - Multiply the counted number by 10,000 to get the number of RBCs per microliter (µL) of blood.
2. **Count White Blood Cells (WBCs):**
   - Focus on the four large corner squares.
   - Count all the cells within these squares.
   - Multiply the counted number by 50 to get the number of WBCs per microliter (µL) of blood.

6. Calculations

1. RBC Count Calculation:

$$\text{RBC count} = \left(\frac{\text{Number of RBCs counted in 5 squares}}{5} \right) \times 10,000$$

2. WBC Count Calculation:

$$\text{WBC count} = \left(\frac{\text{Number of WBCs counted in 4 squares}}{4} \right) \times 50$$

Automatic Optical Method:

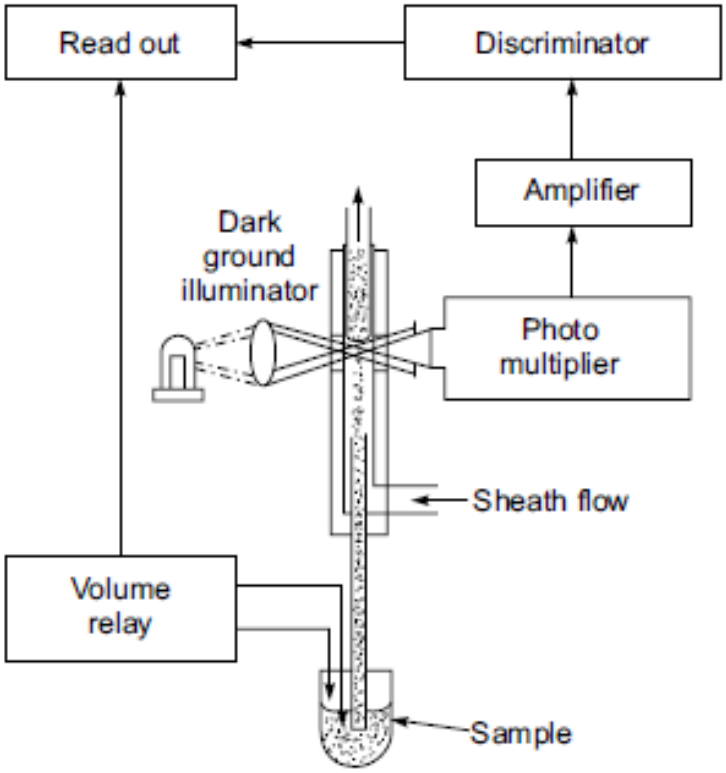

Fig. 5.8.1 Optical method of counting cells

- The method is based on collecting scattered light from the blood cells and converting it into electrical pulses for counting.
- Fig. 5.8.1 shows one type of arrangement for the rapid counting of red and white cells using the optical detection system.
- A sample of dilute blood (1:500 for white cells and 1:50,000 for red cells) is taken in a glass container.
- It is drawn through a counting chamber in which the blood stream is reduced in cross-section by a concentric high velocity liquid sheath.
- A sample optical system provides a dark field illuminated zone on the stream and the light scattered in the forward direction is collected on the cathode of a photomultiplier tube.
- Pulses are produced in the photomultiplier tube corresponding to each cell.

- These signals are amplified in a high input impedance amplifier and fed to an adjustable amplitude discriminator.
- The discriminator provides pulses of equal amplitude, which are used to drive a digital display.
- Instruments based on this technique take about 30 s for completing the count
- The instruments require about 1 ml of blood sample.

Electrical Conductivity Method :

- Blood cell counters, operating on the principle of conductivity change, which occurs each time a cell passes through an orifice, are generally known as ***Coulter Counters***. The method was patented by Coulter in 1956.
- The underlying principle of the measurement is that blood is a poor conductor of electricity whereas certain diluents are good conductors. For a cell count, therefore, blood is diluted and the suspension is drawn through a small orifice.
- By means of a constant current source, a direct current is maintained between two electrodes located on either side of the orifice.
- As a blood cell is carried through the orifice, it displaces some of the conductive fluid and increases the electrical resistance between the electrodes.
- A voltage pulse of magnitude proportional to the particle volume is thus produced. The resulting series of pulses are electronically amplified, scaled and displayed on a suitable display.
- A constant current is normally passed between the electrodes E1 and E2. Therefore, the electric resistance of the liquid measured between these two electrodes changes rapidly when a particle having electric conductance differing from the conductance of the electrolyte passes through the aperture.

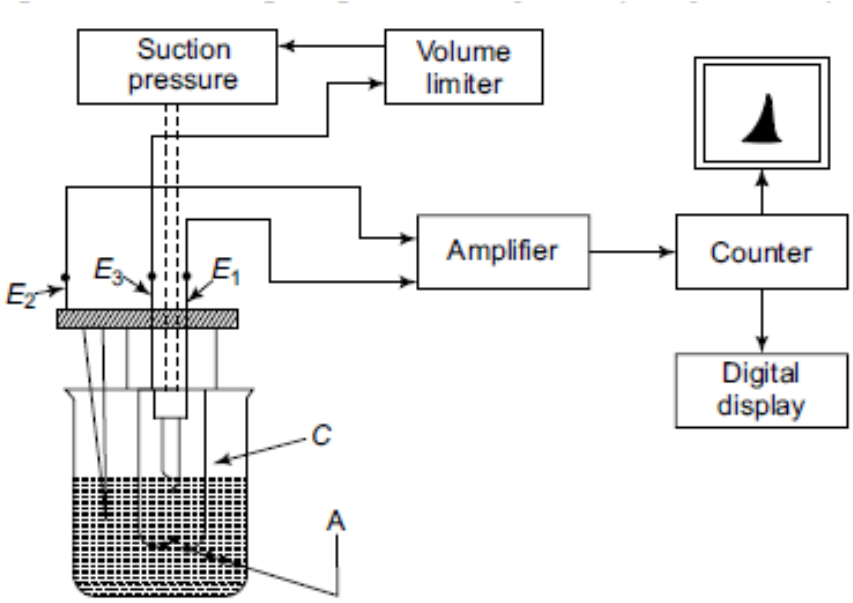

Fig. 5.8.2 Block diagram of blood cell Coulter counter

- This results in the generation of a voltage pulse, which is amplified in a preamplifier of high gain and low noise level.
- The output signal of this stage goes to a discriminator, which compares the amplitude of the pulse arriving at its input with the preset triggering level.
- If the input signal exceeds the triggering level, the discriminator gives out a pulse of constant shape and amplitude.
- These pulses go to a counting circuit for the display of the measured parameter.

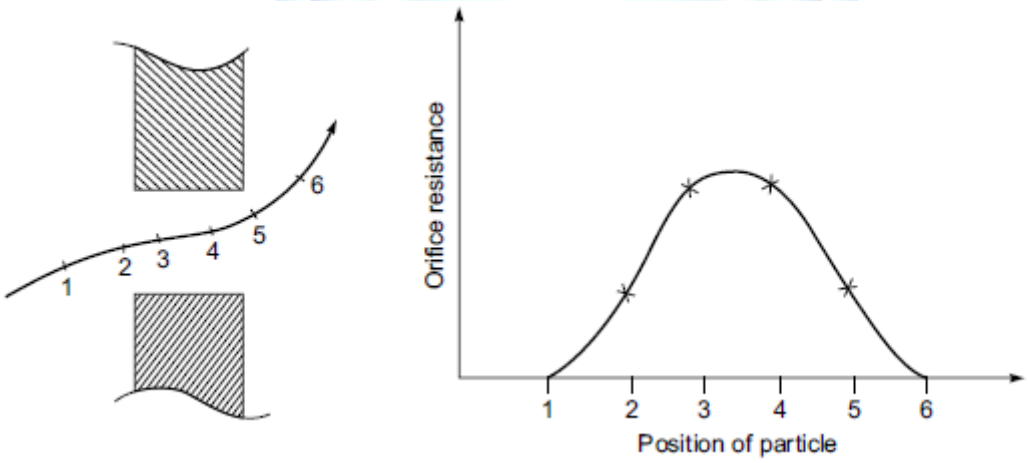

Fig. 5.8.3 The sequence of building up the pulse in terms of increase in resistance at different positions the cell has with respect to the orifice

- Fig. 5.8.3 shows the sequence of building up the pulse in terms of increase in resistance at different positions of the cell with respect to the orifice.
- One great advantage of this instrument is that the clogging of the capillary is greatly eliminated by applying a bi-directional flow during the measurement procedure.
- The number of particles N in a unit volume is determined from the relation,

$$N = \frac{HLE}{V}$$

where

H = factor of dilution
L = scaling factor of the counter
V = measured volume
E = result displayed on the digital display.

- The Coulter counters are usually provided with an LCD to display the pulse information, which has passed through the amplifier, and acts as a visible check on the counting process indicating instantaneously any malfunctions such as a blocked orifice.
- **Errors in Electronic Counters**

  There are a number of errors that may occur in the electronic cell counting technique. Briefly, these errors are categorized as follows:

  - ✓ Aperture Clogging:
  - ✓ Uncertainty of Discriminator Threshold
  - ✓ Coincidence Error
  - ✓ Settling Error
  - ✓ Error in Sample Volume
  - ✓ Error due to Temperature Variation
  - ✓ Biological Factors
  - ✓ Dilution Errors
  - ✓ Error due to External Disturbances

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DEPARTMENT OF BIOMEDICAL ENGINEERING

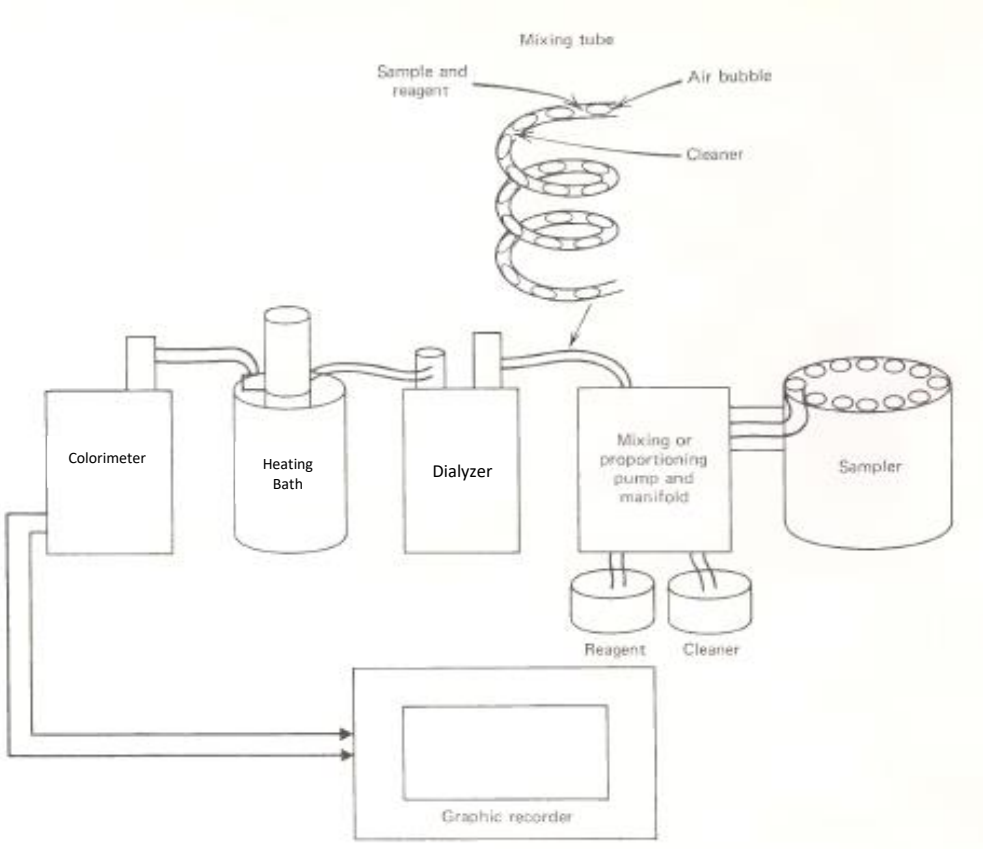
BM3491 Biomedical Instrumentation

UNIT- V BIOCHEMICAL MEASUREMENTS

5.9 Autoanalyzer

An autoanalyzer, also known as an automated analyzer, is a laboratory instrument used for the automatic analysis of chemical or biological samples. These devices are widely used in various fields, including medical diagnostics, environmental monitoring, food and beverage testing, and pharmaceutical research.

The autoanalyzer sequentially measures blood chemistry and displays this on a graphical readout. As shown in Figure 5.9.1, this is accomplished by mixing, reagent reaction, and colorimetric measurement in a continuous stream. The system includes the following elements:



1. Sampler—aspirates samples, _ stan-dards, and wash solutions to the autoanalyzer system.

2. Proportioning pump and manifold

introduces (mixes) samples with reagents to effect the proper chemical color reaction to be read by the colorimeter. It also pumps fluids at precise flow rates to other modules, as proper color development depends on re-action time and temperature.

3. Dialyzer—separates interfacing sub-stances from the sample material by permitting selective passage of sample components through a semipermeable membrane.

4. Heating bath—heats fluids continuously to exact temperature (typically 37°C incubation equivalent to body temperature). Temperature is critical to color development.

5. Colorimeter—monitors the changes in optical density of the fluid stream flowing through a tubular flow cell. Color intensities (optical densities) proportional to substance concentrations, are converted to equivalent electrical voltages.

6. Recorder—converts optical density electrical signal from the colorimeter into a graphic display on a moving chart. The heart of the autoanalyzer system is the proportioning pump. This consists of a peristaltic (occluding or roller) pump. Air segmentation in the mixing tube separates the sample/reagent mixture from the cleaning fluid and other samples. As these air-separated fluids traverse the coil of the mixing tube, effective mixing action is achieved. The Technicon SMA 12/60, shown in Figure 16-20, is a sequential multiple analyzer that performs 12 different tests on 60 samples per hour. It is a continuous flow process that produces a chemical profile read on a graphic chart. Tests accomplished include most of those shown in Table 16-1.

- ✓ A later computerized version is developed.
- ✓ This is the Technicon SMAC. Up to 40 different tests can be performed on an individual serum sample.

- ✓ One problem with automatic analyzers is certain identification of samples. Patient data can be intermixed with other patients if care is not taken. Sterilization is also needed for samples, glassware, and equipment parts that are contaminated with disease. Diseases
- ✓ such as hepatitis or other communicable infections can be spread to equipment operators.
- ✓ Figure shows an autoclave unit used to sterilize small and large items. It operates at saturated steam pressures and temperatures of 120°C for 20 min to one hour. Maintenance on autoanalyzers include frequent calibration adjustment. Most prob-lems are mechanical (tubes, moving pump parts) and electrical (switches, motors).
- ✓ Electronic failures are few. Sophisticated autoanalyzer system maintenance and repair requires that the BMET have gone through manufacturer's schools. Operation and ser-vice manuals must always be consulted. A patient's life may hinge on accurate measurement results obtained by clinical instrumentation.

Maintenance:

Maintenance on autoanalyzers include frequent calibration adjustment. Most prob-lems are mechanical (tubes, moving pump parts) and electrical (switches, motors). Electronic failures are few. Sophisticated autoanalyzer system maintenance and repair requires that the BMET have gone through manufacturer's schools. Operation and ser-vice manuals must always be consulted. A patient's life may hinge on accurate measurement results obtained by clinical instrumentation.

Benefits:

- **Efficiency**: Reduces turnaround time for test results.
- **Consistency**: Minimizes variability in results due to human factors.
- **Cost-Effectiveness**: Decreases labor costs and reagent use through optimized processes.
- **Safety**: Reduces the handling of hazardous substances by laboratory personnel.

Applications:

- **Medical Diagnostics**: Routine blood tests, metabolic panels, hormone levels, etc.
- **Pharmaceuticals**: Drug development and quality control.
- **Environmental Monitoring**: Detection of pollutants and toxins.
- **Food and Beverage Industry**: Ensuring safety and quality of products.

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